

## **Effects of 2,4-D on Callus Induction from Leaf Explants of *Cornukaempferia larsenii* P. Saensouk**

P. SAENSOUK, P. THEERAKULPISUT, B. KIJWIJAN  
AND S. BUNNAG

Applied Taxonomic Research Center, Department of Biology, Faculty of Science,  
Khon Kaen University, Khon Kaen 40002, Thailand.

Email addresses: saensouk@yahoo.com, piythe@kku.ac.th,  
bookij@kku.ac.th, sumbun@kku.ac.th

### **Abstract**

Callus was induced from young leaves of *Cornukaempferia larsenii* P. Saensouk on Murashige and Skoog medium supplemented with 3% sucrose and various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) in light and dark conditions. The highest number of callus formation, percentage of callus formation and average weight of callus were obtained from young leaves cultured on the medium supplemented with 0.5 mg/l 2,4-D in the light condition. The callus could not be regenerated to plantlets in media added with various concentrations of NAA and BA.

### **Introduction**

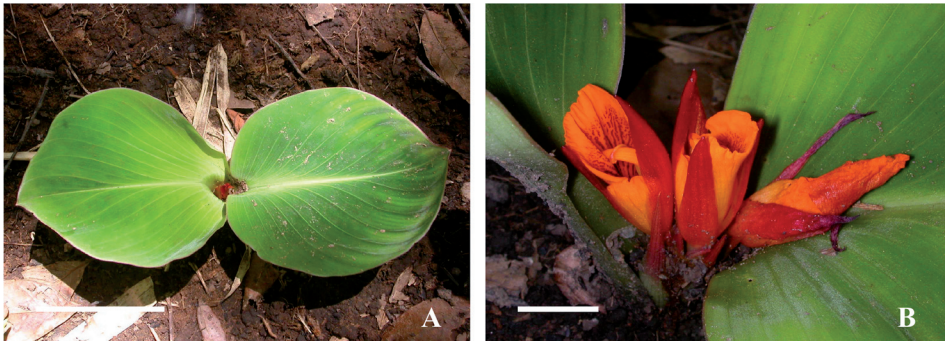
The genus *Cornukaempferia* Mood & K. Larsen is the new genus in Zingiberaceae from Thailand, described by Mood and Larsen (1997, 1999). Two species, *C. aurantiflora* Mood & K. Larsen and *C. longipetiolata* Mood & K. Larsen, have been recognized. This genus is listed as rare and endemic to Thailand and its distribution is restricted to only few provinces in the northeastern and northern part of the country. *C. aurantiflora* has been used by local people in northeastern Thailand to treat infected hemorrhoids and laryngitis common in Thai children. During a floristic survey carried out in May of 2005, a morphologically distinct species of *Cornukaempferia* was discovered and will be named *C. larsenii* in honor of Professor Kai Larsen, University of Aarhus, Denmark (Saensouk *et al.*, 2007).

The new species is propagated vegetatively by pieces of rhizomes. In a vegetatively propagated plant like *Cornukaempferia*, the risk of systemic infections with rootknot nematodes, bacterial wilt and *Fusarium* from the propagules is very high. Thus, the application of tissue culture can be used to produce large amount of disease-free plantlets. The objective of this work

is to establish a system for vegetative propagation of this rare plant species through tissue culture. This is the first report of callus induction from leaf tissue of plants in this genus.

### Materials and Methodology

Young leaves of *C. larsenii* (Fig. 1) collected from natural habitats were washed with running tap water, rinsed with 70% (v/v) ethyl alcohol for 30 seconds, sterilized with 0.9 % sodium hypochlorite containing 2 drops of Tween 20 for 15 seconds followed by three washes with sterilized distilled water. The young leaves were cut into 1x1 cm<sup>2</sup> pieces and cultured on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.7% agar, and 0, 0.1, 0.5, 1, 2, 3, 4 and 5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) in both light and dark conditions for 16 weeks. Callus was transferred to regeneration medium, i.e., MS medium, added with 0, 1, 3 and 5 mg/l 1- naphthaleneacetic acid (NAA) and benzyladenine (BA) for 16 weeks. The cultures were incubated at 25 ± 2 °C under white, fluorescent light (2,000 lux) at a 16 h photoperiod or in the dark. All the experiments were conducted using complete randomized design (CRD) with 15 replicates each containing one explant per culture tube. Data were analyzed using ANOVA and the mean separation was achieved by the Duncan's Multiple Range Test (DMRT). The test of statistical significance was performed at the 5% level using the SPSS program (version 11.5).

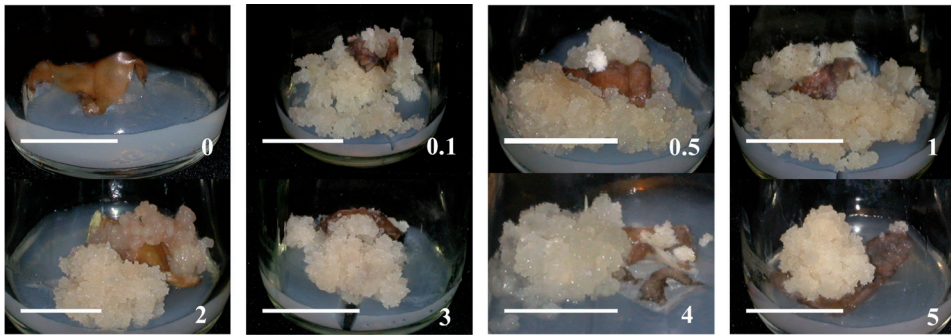


**Figure 1.** *Cornukaempferia larsenii*. A. Habit; B. Flower. (Scale bars A= 10 cm and B= 1 cm).

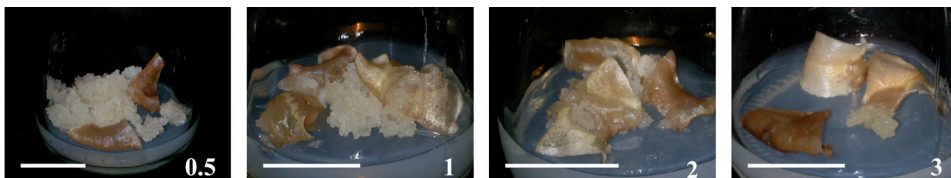
### Results and Discussion

Preliminary efforts to propagate this plant by culturing shoot tips from underground stems resulted in extremely high level of contamination, therefore, an attempt was made using the leaf blade as explants. Young leaves from natural habitats were cultured on MS medium with various levels of

2,4-D for induction of callus in the light and dark conditions. The callus was soft in texture, friable in structure, and yellowish white (Figs. 2 and 3). Callus did not form on medium lacking 2,4-D. The callus formation occurred on medium added with 0.1, 0.5, 1, 2, 3, 4 and 5 mg/l 2,4-D in the light, and 0.5, 1, 2 and 3 mg/l 2,4-D in the dark, after 8 weeks of culture. The highest percentage of callus formation (99.33%) and the highest average weight of callus (2.61 g) were obtained from young leaves cultured on the medium supplemented with 0.5 mg/l 2,4-D in the light condition for 16 weeks (Table 1). These results differ from that of Babu *et al.* (1992) who reported callus formation on the young leaves of *Zingiber officinale* Rosc. (ginger) cv. Maran cultured on MS medium containing 2-5 mg/l 2,4-D. Kackar *et al.* (1993) induced callus formation from young leaf segments of ginger on MS medium added with dicamba. Samsudeen *et al.* (2000) induced ginger anther to develop callus on MS medium supplemented with 2-3 mg/l 2,4-D. Prakash *et al.* (2004) obtained semi-friable callus from leaf sheath explants of *Curcuma amada* Roxb. on MS medium added with 2 mg/l 2,4-D. Moreover, Salvi *et al.* (2001) induced callus from leaf base of turmeric on MS medium supplemented with 2 mg/l dicamba, 2 mg/l picloram or 5 mg/l NAA in combination with 0.5 mg/l BA. Callus was induced more effectively in the light than in the dark condition. These results differ from Malamug *et al.* (1991) who reported callus induction from shoot tips of ginger on MS medium containing 1 mg/l BA and 0.5 mg/l 2,4-D in the dark condition. High contamination of cultures was reported when rhizomes or vegetative buds are used as explants for initiation of culture. By using the leaf tissue as explants this problem was eliminated almost completely. In *Cornukaempferia*, 2,4-D was used for induction of callus from leaf explants (see Table 1), but when callus was transferred to MS medium added with varying concentrations of NAA and BA and cultured for 16 weeks, plant regeneration failed. Varying types and concentrations of auxin and cytokinin have been successfully used to regenerate plantlets from calli of several other species of Zingiberaceae. In ginger, Malamug *et al.* (1991) reported plant regeneration from shoot tip callus on MS medium added with 1 and 3 mg/l 2,4-D. Callus could also be regenerated from the young leaf explants of ginger on MS medium supplemented with 0.2 mg/l 2,4-D and 5 mg/l kinetin or 5 mg/l BA (Babu *et al.*, 1992). Samsudeen *et al.* (2000) was able to regenerate plantlets from callus of ginger anther on MS medium supplemented with 5-10 mg/l BA and 0.2 mg/l 2,4-D. Prakash *et al.* (2004) cultured semi-friable callus from leaf sheath explants of *Curcuma amada* Roxb. on MS medium containing 2 mg/l BA and 0.5 mg/l NAA and produced optimum shoot initiation and development. In addition, Salvi *et al.* (2001) transferred callus of turmeric (*Curcuma longa* Linn.) to half strength MS medium supplemented with 5 mg/l BA in combination with TIBA or 0.1 mg/l 2,4-D, green shoot primordia were seen to differentiate



**Figure 2.** Callus induction from leaf explants of *Cornukaempferia larsenii* on MS medium added with 0, 0.1, 0.5, 1, 2, 3, 4 and 5 mg/l 2,4-D in the light condition (scale bars = 2.5 cm).



**Figure 3.** Callus induction from leaf explants of *Cornukaempferia larsenii* on MS medium added with 0.5, 1, 2 and 3 mg/l 2,4-D in the dark condition (scale bars = 2.5 cm).

**Table 1.** Effect of 2,4-D on callus induction from leaf explants of *Cornukaempferia larsenii* in the light and dark conditions for 16 weeks.

2,4-D (mg/l)	No. of explants	Light			Dark		
		No. of callus formation	% of callus formation	Average weight of callus (g) mean±SE	No. of callus formation	% of callus formation	Average weight of callus (g) mean±SE
0	15	0 a*	0	0 a*	0 a*	0	0 a*
0.1	15	3 ab	20	2.13±0.25 ab	0 a	0	0 a
0.5	15	14 d	93.33	2.61±0.40 d	4 b	26.67	2.20±0.29 b
1	15	11 cd	73.33	2.31±0.48 cd	3 ab	20	1.77±0.25 ab
2	15	8 bc	53.33	2.10±0.36 bc	3 ab	20	1.40±0.40 ab
3	15	7 bc	46.67	2.16±0.35 bc	2 ab	13.33	1.00±0.14 ab
4	15	7 bc	46.67	2.06±0.38 bc	0 a	0	0 a
5	15	6 bc	40	1.95±0.40 b	0 a	0	0 a

\*In each column the values with the different letters differ significantly ( $P = 0.05$ ) as determined by DMRT (see text).

from the surface of the callus. On transfer of regenerating cultures to half MS media supplemented with kinetin, shoot primordia developed into well-differentiated shoots. When shoots were transferred to medium devoid of phyto-hormone, complete rooted plants were obtained. Further experiments are being performed to obtain efficient plant regeneration using different growth regulators and culture conditions.

### Conclusion

This is the first report describing tissue culture of *Cornukaempferia larsenii*, a recently discovered and rare species of Thailand. We reported a successful protocol for the efficient and reliable callus induction using cultured leaf explants of this species. Plant regeneration from leaf tissue through an intermediary callus phase may be a possibility of increasing rate of somaclonal variations that can be exploited for crop improvement which are not available by conventional methods. Furthermore, regeneration of plantlets from callus is an important technique, which can be utilized in the application of tissue culture in developing new germplasm.

### Acknowledgements

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